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ISOLATION AND SCREENING OF LIPASE PRODUCING BACTERIA FROM OIL MILLEFFLUENT

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ABSTRACT

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) are hydrolysis enzyme. They hydrolyze the carboxylic ester bond through various important reactions such as trans-esterification, alcoholysis, interesterification, esterification, aminolysis. The current studyincludes isolation and screening of lipase producing bacteria from oil mill effluent. In this study twenty three bacterial isolates were isolated by pour plate technique. They were cultivated on Nutrient Agar plates. For their lipase producing ability isolates were qualitatively screened by Tween 20 plate method. After incubation ten bacterial isolates were shows lipase activity. Positive culture was quantitative screen by titrimetric method.

Keywords: Lipase, Tween 20, Quantitative, Qualitative

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) are hydrolysis enzyme. They hydrolyze the carboxylic ester bond through various important reactions such as trans-esterification, alcoholysis, interesterification, esterification, aminolysis. Microbes produce lipase; they hydrolyze triglycerides into fatty acid, mono and di-glycerides and glycerol with an important physiological role (Houde*et al.,* 2004). Lipases catalyze and hydrolyze ester bond in triglyceride oil and other esters. In the presence of low-water environment they catalyze ester bonds and when presence of sufficient water hydrolysis is occur (Gumel*et al.,* 2011). They are produced by various plants, animal, bacteria, fungi and yeast. (Ulkar and Karaoglu, 2012). Lipase producing microorganisms are found in industrial water, soil contained with oil, oilseeds, coal crest, vegetable oil processing factories, compost blend, decaying food and dairies product (Jacger*et al.,* 1999 and Houde*et al.,* 2004). Lipases are versatile tool for biotechnology. Bacterial lipases are important in various industries. It has applicable in multiple industries such as agrochemical, pharmaceutical, cosmetic and perfume, taste and flavor industries, textile, food and dairy, detergent and surfactant industries, fat and oil, leather and paper production, chemical and waste water treatment (Hasan*et al.,* 2006 and Treichel*et al.,* 2010). Especially lipases are applied for biodiesel production. In biodiesel production lipase enzyme are using for less energy, catalysis in mild reactions and easily recovered glycerol from biodiesel. They are produces many types of oil from biodiesel production such as palm oil, olive oil, sunflower oil and soybean oil (Bajaj *et al.,* 2010).

MATERIALS AND METHODS

Collection of Effluent Sample

Effluent samples were collected in sterilized glass bottles. Glass bottle was first washed with tap water followed with detergent wash. After washing it with detergent, it was again washed with tap water, thoroughly so that no detergent could stick to it. The bottle was dried in a hot air oven and then wrapped in a paper and was later exposed to 70^0C. Such containers were then, at the time of sample collection, rinsed thoroughly with the sample to be collected. Samples were brought to the laboratory immediately after collection. Distance of sampling sites was such that the samples were transported to the laboratory within 30 minutes time.

Isolation of Bacteria

Pour plate method was used for obtaining pure culture. For isolation of bacteria by serial dilution method. 1 ml of effluent sample was added into 09ml sterile water and mixed. The suspension was serially diluted 10^{-1} to 10^{-5} dilution. After dilution 1ml of each dilution was spread on sterile plate and adds Nutrient Agar medium. The inoculated plates were incubated at 37°C for 24 to 48 hours.

Qualitative Screening of Lipolytic Bacteria by Plate Assay Method

Lipolytic activity of isolates was tested qualitatively by plate test method of (Sierra, 1957) sorbitanmonolaurate (Tween 20) was used as lipid substrate. Tween 20 was sterilized separately by autoclaving for 15 minutes at 121^0C and 1ml was added to 100ml of sterile and cooled basal medium. The medium

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containing plates were point inoculated with the isolated bacteria's and incubated for 2 to 3 days at 35 ± 2^0C. Lipoytic activity was examined by the zone of precipitate formed around a colony due to crystals of calcium salts of lauric acid liberated by the enzyme.

Quantitative Estimation of Lipase Production by Titrimetric Method

Bacteria isolated through qualitative screening were assayed for their potential as lipase producers. Extracellular lipase activity of all the isolated bacteria were assayed by titrimetric method.

The enzyme was assayed by estimating the amount of free fatty acid released in the reaction according to the method of Samad*et al* (1989). The site of action of lipase is the interface between the oil drops and the aqueous phase, so that degree of emulsification plays an important role in establishing the active substrate concentration. A stabilized olive oil emulsion was used as substrate and liberated free fatty acid was determined. "One unit of lipase activity is defined as one micro mole of fatty acid liberated per minute at 45^0C.

Identification of Bacteria

Bacterial cultures were identified on the basis of their morphological and biochemical characters (Aneja, 1993; Dubey&Maheshwari, 2002).Identification was done onmorphological and biochemical characters andwith the help of Bergey's Manual of Systematic Bacteriology (1984, 1986,and 1989) and probabilistic identification kit. Probabilistic identification kit is a computer programming prepared in the laboratory of DrAnjana Sharma, School of Biological Sciences, R.D. University, Jabalpur (M.P). This kit has been named as Probabilistic Identification of Bacteria (PIB) kit. The programming was based on different positive and negative results obtained with various tests and as prescribed by different authorities, for the identification of different species feeding of the data to computer matches the similarity or dissimilarity of the test results of unknown organism with the known organism.

RESULTS AND DISCUSSION

This study mainly based on isolation and screening of lipase producing bacteria from oil mill effluent. Thirteen bacteria colonies were isolated from the sample. Nutrient Agar medium were used for maintain of bacterial isolates. They were screened for their potential lipase producing ability using Twee 20 as a substrate. All bacterial isolates were inoculated in Agar plates which contain 1% Twee 20 and plates were incubated at 37^0C for 24 to 48 hours. The results of screening tests are given in Table ,1.ten bacterial isolates shows positive reaction with had shown Negative reaction.

Thirteen bacteria were thenidentified with the help of Probabilistic identification kit and Bergeys Manual of Systematic Bacteriology. The identified bacteria were*Vibrio parahemolyticus*, *Aeromonashydrophila*, *Micrococcus lilac*, *Branhmella sp.*, *Vibrio vulvificus*, *Pseudomonas aeruginosa*, *Acinetobactercalcoaceticus*, *Neisseria pharynges*, *Lactobacillus brevis*, *Providensiapseudomallei*, *Lactobacillus viridescens*, *Micrococcus luteus 4* and *Obesumbacteriumproteusbiogrop* .

Table 1: Lipase activity of different sp. of bacteria

S.N	Name of bacteria	Lipase activity
1	*Vibrio parahemolyticus*	+
2	*Aeromonashydrophila*	+
3	*Micrococcus lilac*	+
4	*Branhmellasp*	-
5	*Vibrio vulvificus*	+
6	*Pseudomonas aeruginosa*	+
7	*Acinetobactercalcoaceticus*	+
8	*Neisseria pharynges*	+
9	*Lactobacillus brevis*	+
10	*Providenciapseudomellai*	+
11	*Lactobacillus viridescens*	+
12	*Micrococcus luteus 4*	-
13	*Obesumbacteriumproteusbiogrop 1*	-

CONCLUSION

Lipase production was studied in the oil mill effluent bacterial culture. So all the 13 bacteria were screened for lipase production in which 10 bacteria showing lipase production and 3 bacteria cannot produce lipase.

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